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Micropropagation (Shoot initiation and Development) of Hibiscus from nodal explants using BAP (6-Benzyl Amino Purine)

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Abstract

In this study, the nodal explants collected from the healthy shoots were cultured on the basal MS medium supplemented with 3mg/lit of BAP (6-Benzyl Amino Purine). In the earlier study, several researchers reported that the concentration of 3mg/lit is optimum for the growth and development of shoot tip in Hibiscus by the duration of 1month. The different stage of shoot development was observed from the third day to one month interval. It revealed that the callus initiation starts from the 10th day and auxiliary shoot initiation from 2 weeks, and subsequent growth and development was observed from the third week and one month interval respectively. Further the developed healthy shoot culture will be maintained and will be treated with NAA at different concentration for root initiation. Finally, the optimum concentration of NAA will be fixed for better root initiation and development

Key Words: Micropropagation of Hibiscus

Introduction

Hibiscus rosa sinensis belongs to the genus Hibiscus is most familiar flower and suitable for both the tropical and sub-tropical regions of the world. Hibiscus is a profuse flowering woody shrub in united states (Pfeil et al., 2002). The main constraints in commercial production of hibiscus rosa sinensis is pathogenic invasion caused by the nematodes. Although hibiscus limited to the fact that it blooms for only few days, but it has a high commercial value in landscaping. However, hibiscus received most attention in the field of horticulture in term of micropropagation.

The rate of shoot development in case of woodyu species is low and tedious (Perez et al 1997). Furthermore, the browning of culture media is a serious concern in micropropagation of hibiscus rosa sinensis (Jordan et al., 1998; Kotsias and Roussos, 1999). Therefore, it is important to standardize the micropropagation protocol for woody plant species for their utilization (Rasai et al., 1994). However, very few studies have been exhibited in the family Malvaeae. In this study, we supplemented the BAP (6-Benzyl Amino Purine) growth factor with the basal MS medium at the concentration of 3mg/lit. In our previous study we have used the same concentration in rose and we developed the shoot. Hence, we use the same concentration to validate the BAP concentration on Hibiscus rosa sinensis.

Materials and Methods

Plant material

Five Nodal explants processing a lateral bud is selected from the healthy Hibiscus plant and were used as plant material for analyzing the BAP (6- Benzyl amino purine) concentration for shoot initiation through micropropagation.

Sterilization technique

The collected explants were initially washed with tap water for 10-15 minutes. After washing with the tap water, the explants were treated with the liquid detergents for 10 minutes and then washed with tap water three to five times to remove the residues of liquid detergents used. Further, the explants were treated with 70% ethanol for 15 – 30 seconds and washed with sterile water two to three times. Finally, 0.1 % of mercuric chloride was treated for 2-5 minutes and washed with sterile water two to three times.

Culture Media preparation

Culture Media Preparation used in this study is Murashige and Skoog's medium (MS medium) (1962) in semi-solid form. The chemical composition used in the preparation of the MS medium is given in Table 1.

Table 1. Chemical Components and its composition of the MS medium

Components	Volume (mg/lit)	Components	Volume (mg/lit)	Components	Volume (mg/lit)
Macronutrients		**Micronutrients**		**Vitamins**	
NH_4NO_3	1650	KI	0.830	Thiamine	0.100
KNO_3	1900	H_3BO_3	6.20	Pyridoxine	0.500
$CaCl_2.H_2O$	440	$MnSO_4.H_2O$	15.60	Nicotinic Acid	0.500

$MgSO_4$. $7H_2O$	370	$ZnSO_4.7H_2O$	8.60	Myo-Inositol	100
KH_2PO_4	170	$Na_2MoO_4.2H_2O$	0.250		
		$CuSO_4.5H_2O$	0.250		
		$CuSO_4.6H_2O$	0.025		
		$FeSO_4.7H_2O$	27.80		
		Na_2 EDTA	37.30		

Final semi-solid media preparation

All the chemicals were added in double distilled water. To solidify the medium agar-agar was added to the media. Finally, the pH of the media was checked to be 5.8, and boiled the content until it attains homogeneity. In addition, BAP at 3mg/lit was supplemented with the basal MS medium.

Instruments required

All the inoculation practices were conducted in a Laminar air flow chamber, which is the most convenient and reliable thing to maintain the aseptic condition throughout inoculation. In this, all the contaminants were blown away by the ultra-clean blower thereby creating the aseptic condition. During media preparation, a Ph meter was used to check and maintain the optimum level of pH of the medium. An autoclave was used to sterilize the media, glass instruments, etc., For drying the washed glass instruments, a Hot air oven is used. Other instruments viz., Pipettes, Test tubes, Scalpels, Cotton plugs, Scissors, Forceps, Conical flask, Beaker, measuring cylinder, and Petri dish were used for the sterilization and inoculation of the Hibiscus explants.

Results and Discussion

In our study, we have taken five hibiscus explants and inoculated the explants in the basal MS media supplemented with the BAP (6 – Benzyl Amino Purine) at the concentration of 3 mg/lit. observations were recorded from the third day on all the five explants under study. On the third day, the number of explants evaluated was five. Among the five explants, two were contaminated and rejected. There is no growth on the third day was recorded. After one week, all the three explants survived. Also, no growth was found in the three healthy explants. Callus initiation was started after 10 days and was observed on all the three explants. Further, Auxillary shoot initiation was noticed after two weeks. Subsequently, the healthy well-established explants were maintained carefully and we noticed shoot growth after 20 days intervals. Eventually, shoot growth and shoot development takes place after three weeks and one-month interval respectively (Table 2 and Figure 1). However, there is no elongation and multiplication of shoots were found until one month. Further, the explants are allowed for two months to grow and in the future, the well-established

shotted explants will be subjected to rooting media containing (MS + NAA) at different concentrations and will be evaluated for the best concentration on root development. Similar results were recorded by Kumari and Pandey, 2011; Bhalla et al., 2009. The recovery percentage of the five plants revealed that 60 % survival from our study (Table 3).

Table 2. Observation of the growth of the explants at various intervals

S. No	Days of observation	No of explants evalauted	No of explants rejected	Growth status
1.	3rd day	Five	Two	No Growth
2.	1 week	Three	-	No Growth
3.	10 days	Three	-	Callus Initiation
4.	2 weeks	Three	-	Auxillary Shoot Initiation
5.	20 days	Three	-	Shoot growth
6.	3 weeks	Three	-	Shoot development
7.	1month	Three	-	Shoot development

Table 3. Recovery percentage of the explants

S. No	No of Explant	Infected	Recovery Percentage (%)
1.	1	Infected	
2.	2	Not Infected	
3.	3	Not Infected	60%
4.	4	Infected	
5.	5	Not Infected	

Conclusion

In this study, we have conducted an experiment to validate the BAP (6- Benzyl Amino Purine) at 3 mg/lit on micropropagation of Hibiscus rosa sinensis using a shoot tip. From the above results, it is concluded that a 3 mg / lit concentration of BAP is optimum for the proliferation of Hibiscus rosa sinensis shoot culture. Controversy, higher and lower concentration of BAP diminishes the growth and development of shoot tip. Hence, 3mg/ lit of BAP is optimum for shoot tip growth. Further, we maintain the culture and it will be subjected to NAA for root initiation to evaluate the best concentration of the rooting hormone.

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